

## CLAIMS:

1. A gene cassette comprising a combination of genes which, in an appropriate strain background, are able to direct the synthesis of mycaminose or angulosamine and to direct its subsequent transfer to an aglycone or pseudoaglycone.  
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2. A gene cassette according to claim 1, comprising a combination of genes able to direct the synthesis and transfer of mycaminose, wherein:
  - a) at least one of the genes is selected from the group consisting of: *angorf14*, *tylMIII*, *tylMI*, *tylB*, *tylAI*, *tylAII*, *tylla*, *angAI*, *angAII*, *angMIII*, *angB*, *angMI*, *eryG* and *eryK*; and,
  - 10 b) at least one of the genes is a glycosyltransferase gene selected from the group consisting of *tylMII*, *angMII*, *desVII*, *eryCIII*, *eryBV*, *spnP*, and *midI*.
3. A gene cassette according to claim 2, wherein one of the genes within the gene cassette is *tylla*  
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4. A gene cassette according to claim 2, wherein one of the genes within the gene cassette is *angorf14*
5. A gene cassette according to claim 2 or 4, which comprises *angAI*, *angAII*, *angorf14*, *angMIII*, *angB* and *angMI*, in combination with one or more glycosyltransferase genes selected from the group consisting of *eryCIII*, *tylMII* and *angMII*.  
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6. A gene cassette according to claim 2 or 3, which comprises *tylAI*, *tylAII*, *tylMIII*, *tylB*, *tylla* and *tylMI*, in combination with one or more glycosyltransferase genes selected from the group consisting of *eryCIII*, *tylMII* and *angMII*.  
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7. A gene cassette according to claim 1 comprising a combination of genes able to direct the synthesis and transfer of angulosamine, wherein:
  - a) at least one of the genes is selected from the group consisting of: *angMIII*, *angMI*, *angB*, *angAI*, *angAII*, *angorf14*, *angorf4*, *tylMIII*, *tylMI*, *tylB*, *tylAI*, *tylAII*, *eryCVI*, *spnO*, *eryBVI*, and *eryK*; and,
  - 30 b) at least one of the genes is a glycosyltransferase gene selected from the group consisting of *eryCIII*, *tylMII*, *angMII*, *desVII*, *eryBV*, *spnP* and *midI*.

8. A gene cassette according to claim 7, which comprises *angMIII*, *angMI*, *angB*, *angAI*, *angAII*, *angorf14* and *spnO*, in combination with one or more glycosyltransferase genes selected from the group consisting of *angMII*, *tylMII* and *eryCIII*.

5 9. A gene cassette according to claim 7, which comprises *angMIII*, *angMI*, *angB*, *angAI*, *angAII*, *angorf4*, and *angorf14*, in combination with one or more glycosyltransferase genes selected from the group consisting of *angMII*, *tylMII* and *eryCIII*.

10 10. A process for the production of erythromycins and azithromycins which contain either mycaminose or angolosamine at the C-5 position, said process comprising transforming a strain with a gene cassette as described in any one of claims 1-9 above and culturing the strain under appropriate conditions for the production of said erythromycin or azithromycin.

15 11. The process of claim 10, wherein the strain is selected from actinomycetes, *Pseudomonas*, myxobacteria, and *E. coli*.

12. The process of claim 10, wherein the host strain is additionally transformed with the *ermE* from *S. erythraea*.

20 13. The process of claim 10 or claim 11, wherein the host strain is an actinomycete.

14. The process of claim 13, wherein the host strain is selected from *S. erythraea*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces albus*, *Streptomyces lividans*, *Streptomyces hygroscopicus* sp., *Streptomyces hygroscopicus* var. *ascomyceticus*, *Streptomyces longisporoflavus*, *Saccharopolyspora spinosa*, *Streptomyces tsukubaensis*, *Streptomyces coelicolor*, *Streptomyces fradiae*, *Streptomyces rimosus*, *Streptomyces avermitilis*, *Streptomyces eurythermus*, *Streptomyces venezuelae*, and *Amycolatopsis mediterranei*.

30 15. The process of claim 14, wherein the host strain is *S. erythraea*.

16. The process of claim 15, wherein the host strain is selected from the SGQ2, Q42/1 or 18A1 strains of *S. erythraea*.

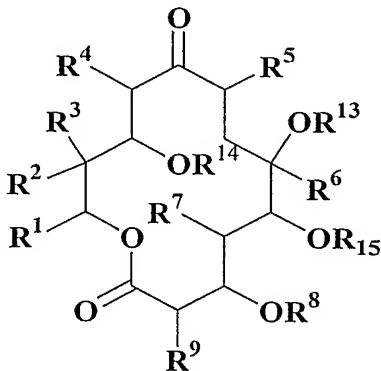
35 17. The process of any one of claims 10 to 16, which further comprises feeding of an aglycone and/or a pseudoaglycone substrate to the recombinant strain.

18. The process of claim 17, wherein said aglycone and/or pseudoaglycone is selected from the group consisting of 3-*O*-mycarosyl erythronolide B, erythronolide B, 6-deoxy erythronolide B, 3-*O*-mycarosyl-6-deoxy erythronolide B, tylactone, spinosyn pseudoaglycone, 3-*O*-rhamnosyl erythronolide B, 3-*O*-rhamnosyl-6-deoxy erythronolide B, 3-*O*-angulosaminyl erythronolide B, 15-hydroxy-3-*O*-mycarosyl erythronolide B, 15-hydroxy erythronolide B, 15-hydroxy-6-deoxy erythronolide B, 15-hydroxy-3-*O*-mycarosyl-6-deoxy erythronolide B, 15-hydroxy-3-*O*-rhamnosyl erythronolide B, 15-hydroxy-3-*O*-rhamnosyl-6-deoxy erythronolide B, 15-hydroxy-3-*O*-angulosaminyl erythronolide B, 14-hydroxy-3-*O*-mycarosyl erythronolide B, 14-hydroxy erythronolide B, 14-hydroxy-6-deoxy erythronolide B, 14-hydroxy-3-*O*-mycarosyl-6-deoxy erythronolide B, 14-hydroxy-3-*O*-rhamnosyl erythronolide B, 14-hydroxy-3-*O*-rhamnosyl-6-deoxy erythronolide B, 14-hydroxy-3-*O*-angulosaminyl erythronolide B.

19. The process of any one of claims 10 to 18, which additionally comprises the step of isolating the compound produced.

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20. A compound according to the formula I below:



R<sup>1</sup> is selected from:

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- H, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>
- an alpha-branched C<sub>3</sub>-C<sub>8</sub> group selected from alkyl, alkenyl, alkynyl, alkoxyalkyl and alkylthioalkyl groups any of which may be optionally substituted by one or more hydroxyl groups;
- a C<sub>5</sub>-C<sub>8</sub> cycloalkylalkyl group wherein the alkyl group is an alpha-branched C<sub>2</sub>-C<sub>5</sub> alkyl group
- a C<sub>3</sub>-C<sub>8</sub> cycloalkyl group or C<sub>5</sub>-C<sub>8</sub> cycloalkenyl group, either of which may optionally be substituted by one or more hydroxyl, or one or more C<sub>1</sub>-C<sub>4</sub> alkyl groups or halo atoms

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- a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C<sub>1</sub>-C<sub>4</sub> alkyl groups, halo atoms or hydroxyl groups
- phenyl which may be optionally substituted with at least one substituent selected from C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkoxy and C<sub>1</sub>-C<sub>4</sub> alkylthio groups, halogen atoms, trifluoromethyl, and cyano or
- R<sup>17</sup>-CH<sub>2</sub>- where R<sup>17</sup> is H, C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>2</sub>-C<sub>8</sub> alkenyl, C<sub>2</sub>-C<sub>8</sub> alkynyl, alkoxyalkyl or alkylthioalkyl containing from 1 to 6 carbon atoms in each alkyl or alkoxy group wherein any of said alkyl, alkoxy, alkenyl or alkynyl groups may be substituted by one or more hydroxyl groups or by one or more halo atoms; or a C<sub>3</sub>-C<sub>8</sub> cycloalkyl or C<sub>5</sub>-C<sub>8</sub> cycloalkenyl either of which may be optionally substituted by one or more C<sub>1</sub>-C<sub>4</sub> alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated or fully or partially unsaturated and which may optionally be substituted by one or more C<sub>1</sub>-C<sub>4</sub> alkyl groups or halo atoms; or a group of the formula SA<sub>16</sub> wherein A<sub>16</sub> is C<sub>1</sub>-C<sub>8</sub> alkyl, C<sub>2</sub>-C<sub>8</sub> alkenyl, C<sub>2</sub>-C<sub>8</sub> alkynyl, C<sub>3</sub>-C<sub>8</sub> cycloalkyl, C<sub>5</sub>-C<sub>8</sub> cycloalkenyl, phenyl or substituted phenyl wherein the substituent is C<sub>1</sub>-C<sub>4</sub> alkyl, C<sub>1</sub>-C<sub>4</sub> alkoxy or halo, or a 3 to 6 membered oxygen or sulphur-containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C<sub>1</sub>-C<sub>4</sub> alkyl groups or halo atoms

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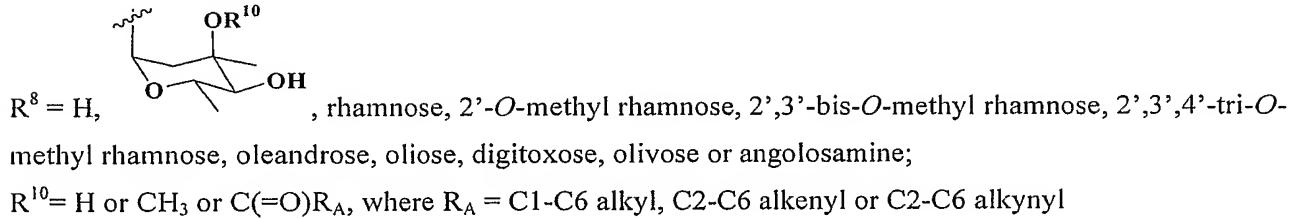
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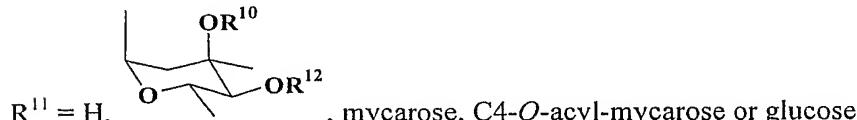
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R<sup>2</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>9</sup> are each independently H, OH, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub> or OCH<sub>3</sub>

R<sup>3</sup>= H or OH



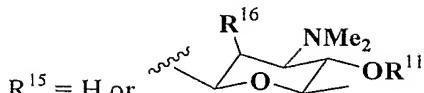
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R<sup>12</sup>= H or C(=O)R<sub>A</sub>, where R<sub>A</sub>= C1-C6 alkyl, C2-C6 alkenyl or C2-C6 alkynyl

R<sup>13</sup>= H or CH<sub>3</sub>



5       $\text{R}^{16} = \text{H}$  or  $\text{OH}$

10       $\text{R}^{14} = \text{H}$  or  $-\text{C}(\text{O})\text{NR}^{\text{c}}\text{R}^{\text{d}}$  wherein each of  $\text{R}^{\text{c}}$  and  $\text{R}^{\text{d}}$  is independently  $\text{H}$ ,  $\text{C}_1\text{-C}_{10}$  alkyl,  $\text{C}_2\text{-C}_{20}$  alkenyl,  $\text{C}_2\text{-C}_{10}$  alkynyl,  $-(\text{CH}_2)_m(\text{C}_6\text{-C}_{10}$  aryl), or  $-(\text{CH}_2)_m(5\text{-}10$  membered heteroaryl), wherein  $m$  is an integer ranging from 0 to 4, and wherein each of the foregoing  $\text{R}^{\text{c}}$  and  $\text{R}^{\text{d}}$  groups, except  $\text{H}$ , may be substituted by 1 to 3  $\text{Q}$  groups; or wherein  $\text{R}^{\text{c}}$  and  $\text{R}^{\text{d}}$  may be taken together to form a 4-7 membered saturated ring or a 5-10 membered heteroaryl ring, wherein said saturated and heteroaryl rings may include 1 or 2 heteroatoms selected from  $\text{O}$ ,  $\text{S}$  and  $\text{N}$ , in addition to the nitrogen to which  $\text{R}^{\text{c}}$  and  $\text{R}^{\text{d}}$  are attached, and said saturated ring may include 1 or 2 carbon-carbon double or triple bonds, and said saturated and heteroaryl rings may be substituted by 1 to 3  $\text{Q}$  groups; or  $\text{R}^2$  and  $\text{R}^{17}$  taken together form a carbonate ring; each  $\text{Q}$  is independently selected from halo, cyano, nitro, trifluoromethyl, azido,  $-\text{C}(\text{O})\text{Q}^1$ ,  $-\text{OC}(\text{O})\text{Q}^1$ ,  $-\text{C}(\text{O})\text{OQ}^1$ ,  $-\text{OC}(\text{O})\text{OQ}^1$ ,  $-\text{NQ}^2\text{C}(\text{O})\text{Q}^3$ ,  $-\text{C}(\text{O})\text{NQ}^2\text{Q}^3$ ,  $-\text{NQ}^2\text{Q}^3$ , hydroxy,  $\text{C}_1\text{-C}_6$  alkyl,  $\text{C}_1\text{-C}_6$  alkoxy,  $-(\text{CH}_2)_m(\text{C}_6\text{-C}_{10}$  aryl), and  $-(\text{CH}_2)_m(5\text{-}10$  membered heteroaryl), wherein  $m$  is an integer ranging from 0 to 4, and wherein said aryl and heteroaryl substituents may be substituted by 1 or 2 substituents independently selected from halo, cyano, nitro, trifluoromethyl, azido,  $-\text{C}(\text{O})\text{Q}^1$ ,  $-\text{C}(\text{O})\text{OQ}^1$ ,  $-\text{OC}(\text{O})\text{OQ}^1$ ,  $-\text{NQ}^2\text{C}(\text{O})\text{Q}^3$ ,  $-\text{C}(\text{O})\text{NQ}^2\text{Q}^3$ ,  $-\text{NQ}^2\text{Q}^3$ , hydroxy,  $\text{C}_1\text{-C}_6$  alkyl, and  $\text{C}_1\text{-C}_6$  alkoxy;

15      each  $\text{Q}^1$ ,  $\text{Q}^2$  and  $\text{Q}^3$  is independently selected from  $\text{H}$ ,  $\text{OH}$ ,  $\text{C}_1\text{-C}_{10}$  alkyl,  $\text{C}_1\text{-C}_6$  alkoxy,  $\text{C}_2\text{-C}_{10}$  alkenyl,  $\text{C}_2\text{-C}_{10}$  alkynyl,  $-(\text{CH}_2)_m(\text{C}_6\text{-C}_{10}$  aryl), and  $-(\text{CH}_2)_m(5\text{-}10$  membered heteroaryl), wherein  $m$  is an integer ranging from 0 to 4; with the proviso that the compound is not 5-*O*-dedesosaminy-5-*O*-mycaminosyl erythromycin A or D

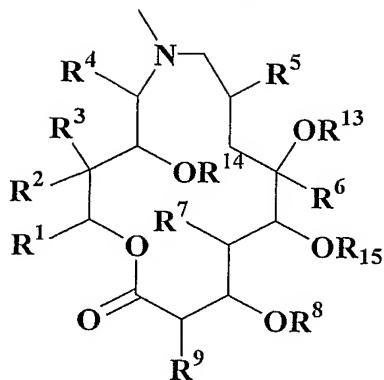
20      or said compound is a variant of any of the above in which the  $-\text{CHOR}^{14}-$  at C11 is replaced by a methylene group ( $-\text{CH}_2-$ ), a keto group ( $\text{C=O}$ ), or by a 10,11-olefinic bond;

25      or said compound is a variant of any of the above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group:  $-\text{CO}-$ ,  $-\text{CH}(\text{OH})-$ , alkene  $-\text{CH}-$ , and  $\text{CH}_2$ ); with the proviso that the compounds are not selected from the group consisting of 5-*O*-dedesosaminy-5-*O*-mycaminosyl erythromycin A and 5-*O*-dedesosaminy-5-*O*-mycaminosyl erythromycin D.

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21.      A compound according to the formula II below:

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$R^1$  is selected from:

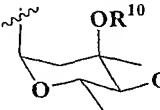
- H,  $CH_3$ ,  $C_2H_5$
- an alpha-branched  $C_3$ - $C_8$  group selected from alkyl, alkenyl, alkynyl, alkoxyalkyl and alkylthioalkyl groups any of which may be optionally substituted by one or more hydroxyl groups;
- a  $C_5$ - $C_8$  cycloalkylalkyl group wherein the alkyl group is an alpha-branched  $C_2$ - $C_5$  alkyl group
- a  $C_3$ - $C_8$  cycloalkyl group or  $C_5$ - $C_8$  cycloalkenyl group, either of which may optionally be substituted by one or more hydroxyl, or one or more  $C_1$ - $C_4$  alkyl groups or halo atoms
- a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more  $C_1$ - $C_4$  alkyl groups, halo atoms or hydroxyl groups
- phenyl which may be optionally substituted with at least one substituent selected from  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  alkoxy and  $C_1$ - $C_4$  alkylthio groups, halogen atoms, trifluoromethyl, and cyano or
- $R^{17}-CH_2-$  where  $R^{17}$  is H,  $C_1$ - $C_8$  alkyl,  $C_2$ - $C_8$  alkenyl,  $C_2$ - $C_8$  alkynyl, alkoxyalkyl or alkylthioalkyl containing from 1 to 6 carbon atoms in each alkyl or alkoxy group wherein any of said alkyl, alkoxy, alkenyl or alkynyl groups may be substituted by one or more hydroxyl groups or by one or more halo atoms; or a  $C_3$ - $C_8$  cycloalkyl or  $C_5$ - $C_8$  cycloalkenyl either of which may be optionally substituted by one or more  $C_1$ - $C_4$  alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated or fully or partially unsaturated and which may optionally be substituted by one or more  $C_1$ - $C_4$  alkyl groups or halo atoms; or a group of the formula  $SA_{16}$  wherein  $A_{16}$  is  $C_1$ - $C_8$  alkyl,  $C_2$ - $C_8$  alkenyl,  $C_2$ - $C_8$  alkynyl,  $C_3$ - $C_8$  cycloalkyl,  $C_5$ - $C_8$  cycloalkenyl, phenyl or substituted phenyl wherein the substituent is  $C_1$ - $C_4$  alkyl,  $C_1$ - $C_4$  alkoxy or halo, or a 3 to 6 membered oxygen or sulphur-containing heterocyclic ring

which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C<sub>1</sub>-C<sub>4</sub> alkyl groups or halo atoms

R<sup>2</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>9</sup> are each independently H, OH, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub> or OCH<sub>3</sub>

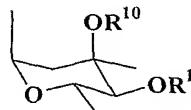
R<sup>3</sup> = H or OH

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R<sup>8</sup> = H, rhamnose, 2'-O-methyl rhamnose, 2',3'-bis-O-methyl rhamnose, 2',3',4'-tri-O-methyl rhamnose, oleandrose, oliose, digitoxose, olivose or angulosamine;

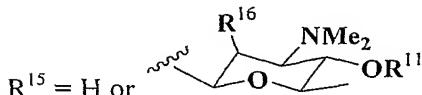
R<sup>10</sup> = H or CH<sub>3</sub> or C(=O)R<sub>A</sub>, where R<sub>A</sub> = C1-C6 alkyl, C2-C6 alkenyl or C2-C6 alkynyl



10 R<sup>11</sup> = H, mycarose, C4-O-acyl-mycarose or glucose

R<sup>12</sup> = H or C(=O)R<sub>A</sub>, where R<sub>A</sub> = C1-C6 alkyl, C2-C6 alkenyl or C2-C6 alkynyl

R<sup>13</sup> = H or CH<sub>3</sub>



15 R<sup>16</sup> = H or OH

R<sup>14</sup> = H or -C(O)NR<sup>c</sup>R<sup>d</sup> wherein each of R<sup>c</sup> and R<sup>d</sup> is independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>20</sub> alkenyl, C<sub>2</sub>-C<sub>10</sub> alkynyl, -(CH<sub>2</sub>)<sub>m</sub>(C<sub>6</sub>-C<sub>10</sub> aryl), or -(CH<sub>2</sub>)<sub>m</sub>(5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4, and wherein each of the foregoing R<sup>c</sup> and R<sup>d</sup> groups, except H, may be substituted by

20 1 to 3 Q groups; or wherein R<sup>c</sup> and R<sup>d</sup> may be taken together to form a 4-7 membered saturated ring or a 5-10 membered heteroaryl ring, wherein said saturated and heteroaryl rings may include 1 or 2

heteroatoms selected from O, S and N, in addition to the nitrogen to which R<sup>c</sup> and R<sup>d</sup> are attached, and said saturated ring may include 1 or 2 carbon-carbon double or triple bonds, and said saturated and heteroaryl rings may be substituted by 1 to 3 Q groups; or R<sup>2</sup> and R<sup>17</sup> taken together form a carbonate

25 ring; each Q is independently selected from halo, cyano, nitro, trifluoromethyl, azido, -C(O)Q<sup>1</sup>, -OC(O)Q<sup>1</sup>, -C(O)OQ<sup>1</sup>, -OC(O)OQ<sup>1</sup>, -NQ<sup>2</sup>C(O)Q<sup>3</sup>, -C(O)NQ<sup>2</sup>Q<sup>3</sup>, -NQ<sup>2</sup>Q<sup>3</sup>, hydroxy, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, -(CH<sub>2</sub>)<sub>m</sub>(C<sub>6</sub>-C<sub>10</sub> aryl), and -(CH<sub>2</sub>)<sub>m</sub>(5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4, and wherein said aryl and heteroaryl substituents may be substituted by 1 or 2 substituents independently selected from halo, cyano, nitro, trifluoromethyl, azido, -C(O)Q<sup>1</sup>, -C(O)OQ<sup>1</sup>, -OC(O)OQ<sup>1</sup>, -NQ<sup>2</sup>C(O)Q<sup>3</sup>, -C(O)NQ<sup>2</sup>Q<sup>3</sup>, -NQ<sup>2</sup>Q<sup>3</sup>, hydroxy, C<sub>1</sub>-C<sub>6</sub> alkyl, and C<sub>1</sub>-C<sub>6</sub> alkoxy;

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 each Q<sup>1</sup>, Q<sup>2</sup> and Q<sup>3</sup> is independently selected from H, OH, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>2</sub>-C<sub>10</sub> alkenyl, C<sub>2</sub>-C<sub>10</sub> alkynyl, -(CH<sub>2</sub>)<sub>m</sub>(C<sub>6</sub>-C<sub>10</sub> aryl), and -(CH<sub>2</sub>)<sub>m</sub>(5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4;

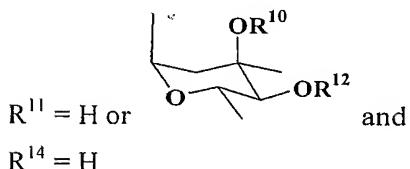
5 or said compound is a variant of any of the above in which the -CHOR<sup>14</sup>- at C12 is replaced by a methylene group (-CH<sub>2</sub>-), a keto group (C=O), or by a 11,12-olefinic bond;

or said compound is a variant of any of the above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: -CO-, -CH(OH)-, alkene -CH-, and CH<sub>2</sub>).

22. A compound according to claim 20 or 21, wherein: R<sup>2</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and R<sup>9</sup> are all CH<sub>3</sub>

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23. A compound according to claim 22, wherein



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24. A compound according to claim 23, wherein R<sup>1</sup> = C<sub>2</sub>H<sub>5</sub> optionally substituted with a hydroxyl group

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25. A compound according to claim 24, wherein R<sup>12</sup> = H

26. A compound according to claim 25, wherein R<sup>1</sup> = C<sub>2</sub>H<sub>5</sub>